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Determination of the Bioconcentration Factor in Some Pesticides by Flow-through Fish Test

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Flow-through fish test를 이용한 일부 농약의 생물농축계수의 측정

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ABSTRACT

The present study was performed to investigate the bioconcentration of methidathion and phosalone. The BCFs (bioconcentration factors), depuration rate constants and LC_{50} for two pesticides in zebrafish (*Brachydanio rerio*) were measured by the flow-through system (OECD guideline 305). The results obtained are summarized as follows : The 24-hr LC_{50}, 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50} were 28.34, 35.98, 24.43, 22.03 for methidathion. The average BCF values of methidathion were 11.25(n=4) and 8.72(n=4) at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(0.022 ㎎/ℓ and 0.22 ㎎/ℓ) after 12 ~72 hrs. The 24-hr LC_{50}, 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50} were 3.76, 2.43, 1.86 and 1.05 ㎎/ℓ for phosalone. The average BCF value of phosalone was 48.88(n=4) at one-hundredth concentration of 96-hr LC_{50}(0.01 ㎎/ℓ) after 12 ~72hrs. However, phosalone was not detected throughout the experimental period at the low concentration(0.001 ㎍/㎖). The LC_{50} value in zebrafish showed that acute toxicity of phosalone was higher than that of methidathion. The BCF values of phosalone were 5 times higher than those of methidathion, and depuration rate of phosalone was faster than that of methidathion.

Keywords : Bioconcentration factor (BCF), Methidathion, Phosalone, Flow-through fish test

요 약

Zebrafish(*Brachydanio rerio*)를 실험어류로 하여 methidathion과 phosalone의 생물농축계수(bioconcentration factor : BCF)와 배설속도상수(depuration rate constant) 및 LC_{50}를 측정하였다. Methidathion의 24, 48, 72, 96시간 LC_{50}는 각각 28.34, 35.98, 24.43, 22.03 ㎎/ℓ로 측정되었다. Methidathion 0.22 ㎎/ℓ (고농도)와 0.022 ㎎/ℓ (저농도)에서 어류 체내에서의 농축정도는 두 농도군에서 각각 12시간 이후에 정류상태에 도달하여 72시간동안 거의 일정하였고, BCF값도 12시간에서 72시간 사이에 고농도와 저농도에서 8.72(n=4)와 11.25(n=4)로 조사되었다. 배설속도상수는 고농도와 저농도에서 6시간 이내에 모두 배설되어 배설속도상수를 구할 수 없었다.

Phosalone의 24, 48, 72, 96시간 LC_{50}는 각각 3.76, 2.43, 1.86, 1.05 ㎎/ℓ로 측정되었다. Zebrafish 체내에서의 농축정도와 BCF값은 고농도(0.01 ㎎/ℓ)에서 12시간 이후에 정류상태에 도달하여 72시간동안 거의 일정하였고, BCF값은 12시간에서 72시간 사이에 48.88(n=4)로 측정되었다. 저농도(0.001 ㎎/ℓ)에서는 실험 전기간동안 zebrafish 체내에서 phosalone이 검출되지 않아 BCF값을 산출할 수 없었다. Zebrafish 체내에서 phosalone(고농도)의 배설속도상수와 반감기를 구하기 위하여 6, 12시간의 배설 실험결과 각각 0.17 hr^{-1}과 4.01시간이었다. Methidathion과 phosalone의 BCF값은 phosalone이 methidathion보다 약 5배 정도 높게 나타났으며, 농약의 배설속도는 phosalone이 methidathion보다 빨랐다.

Ⅰ. Introduction

Water pollution has been increasing due to industrialization and civilization. Pesticides are a widespread pollutant of aquatic ecosystems and may have deleterious effects on living resources. Recently, organophosphorus pesticides, carbamates, pyrethroids and triazines have largely replaced the organochlorine

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compounds in the agricultural practices.

The organophosphorus pesticides methidathion [*S*-(2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl) *O*,*O*-dimethyl phosphorodithioate] and phosalone [*S*-5-chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl *O*,*O*-diethyl phosphorodithioate] are widely used in Korea for pest control such as caterpillar, beetle, scale and lygus.[1)]

Recent studies have demonstrated that organophosphorus insecticides have short and long term effect on survival of vertebrate, tissue accumulation, and on the physiological and reproductive processes of some organisms. Thus, the bioconcentration process of pesticides by aquatic organisms has been extensively studied.

The bioconcentration factor (BCF), which is generally used to estimate the propensity to accumulate chemicals in organisms, is defined as the ratio of the concentration of the chemical in whole fish at steady state to the concentration of the chemical in water during the exposure period.

On the basis of our earlier work on bioconcentration[2~13)], this paper describes the results of the bioconentration and depuration of two organophosphorus insecticides in zebrafish (*Brachydanio rerio*) at two concentration levels under flow through system.[14)]

Ⅱ. Materials and Methods

1. Materials

Methidathion(97% purity) and phosalone(98% purity) were obtained from Kyung Nong corporation in Korea and used without further purification. All solvents used were pesticide residue grade with no further treatment. Sep-Pak Florisil column(Waters, USA) was used for sample purification.

Zebrafish (*Brachydanio rerio*) were purchased from a commercial supplier in Korea, weighed 0.2 to 0.4 g and had an average length of 2.0 to 3.0 cm. All fish were acclimated in glass aquaria containing dechlorinated tap water for at least 4 weeks before use in experiments. Fish were maintained on a 8 : 16hr. dark:light photoperiod. They were fed with commercial balanced fish feed at rate of 1% body weight per day. Excrements and surplus food were removed daily. Methidathion and phosalone were not detected in zebrafish before exposure to these pesticides. The characteristics of experimental water were : temperature, 23.5 ±1℃ ; pH, 7.5 ±0.1 ; DO, 7.1 ±0.1 ㎎/ℓ ; hardness, 38 ±2 ㎎ $CaCO_3$/ℓ .

Gas chromatograph(Shimadzu, Model GC-14A, with a flam photometric detector and a CR-6A data processor) was used in pesticide analysis.

2. Methods

Acute Toxicity Test A static acute toxicity test was performed according to OECD guideline 203 to determine the LC_{50} values of two pesticides.[15)] To determine the LC_{50} values, ten fish in each tank were exposed in five serial concentrations of two pesticides. The fish were not fed for 24 hr. prior to or during the acute toxicity test. The concentrations tested were 15, 20, 25, 30 and 35 ㎎/ℓ for methidathion, 1.0, 2.0, 3.0, 4.0 and 5.0 ㎎/ℓ for phosalone. Dead fish were counted and removed after every 3 hr. through 96 hr. of exposure. The LC_{50} values of each pesticide were determined using a logarithmic probability regression on actual concentrations.

Bioconcentration and Depuration Test Bioconcentration and depuration tests were carried out according to OECD guideline 305 in a continuous flow-through system.[14)]

In the bioconcentration phase at the experiments, zebrafish were maintained at two concentrations of each pesticide for 168 hours. The stock solutions were prepared by dissolving acetone solution (2 ㎖) of methidathion (high exposure level 22 ㎎/ℓ , low exposure level 2.2 ㎎/ℓ) and phosalone (high exposure level 1 ㎎/ℓ , low exposure level 0.1 ㎎/ℓ) with dechlorinated tap water to 10 ℓ , respectively. The solutions were supplied to each of the four glass mixing chambers and connected to peristaltic pumps (Chunse BX-20, Korea) that generated constant solution flow of 3 ㎖/min diluting to the desired concentrations by constant dechlorinated tap water flow of 300 ㎖/min, the outlets were connected to each of the four 100 ℓ glass aquaria containing 250 fish. In this way, the aqueous test solution was diluted 100 times continuously. The concentrations of the

pesticides in each exposure tank were [mean±SD (n=6)] 215.3±15.2 ㎍/ℓ for methidathion (high exposure level), 21.2±1.7 ㎍/ℓ for methidathion (low exposure level), 11.1±1.2 ㎍/ℓ for phosalone (high exposure level) and 1.0±0.1 ㎍/ℓ for phosalone (low exposure level). Test aquaria renewed approximately 4.3 times a day. Zebrafish were exposed to each pesticide for 168 hours.

After 6, 12, 24, 48 and 72 hours, twenty fish were removed, rinsed with distilled water, weighed and analyzed. After the exposure period, fish were transferred to clean water with same flow-through system but without each pesticide. Twenty fish were taken at 6 and 12 hr, respectively.

Analysis Fish samples(ca. 5 g) were placed in a blender jar and anhydrous sodium sulfate (4 g) added. The contents were thoroughly mixed and acetonitrile (30 ㎖) was then added. The mixture was blended at high speed for 4 min. The homogenate was vacuum filtered through a GF/C glass filter. This operation was repeated and combined filtrate was then dried in a rotary evaporator under vacuum at 40℃. The residue was redissolved in 5 ㎖ of hexane and transferred to a preparative Sep-pak florisil column. The column was previously conditioned with 10 ㎖ of hexane and eluted with 20 ㎖ of acetone/hexane(8/2, v/v). The eluate was dried in a rotary evaporator under vacuum at 40℃ and dissolved in 2 ㎖ hexane and analyzed by GC-FPD. Average recoveries(n=3) were 91% for methidathion and 89% for phosalone at a spiked level of 10 ㎍/ℓ.

To evaluate the concentration of each pesticide in the aquaria, 100 ㎖ of test water were collected and extracted with 50 ㎖ of ethyl ether/hexane(4/1, v/v). Extraction with ethyl ether+hexane was repeated and all extracts were combined and passed through glass column with anhydrous sodium sulfate. The eluate was dried in a rotary evaporator under vacuum at 40℃ and dissolved in 2 ㎖ hexane and analyzed by GC-FPD. Average recoveries(n=3) were 95% for methidathion and 92% for phosalone at a spiked level of 1 ㎍/g.

The above GC analysis was performed on a Shimadzu GC-14A with a flame photometric detector. The fused silica capillary column (DB-17, 1 ㎛ thickness, J&W Scientifics) was 30 m by 0.53 ㎜ ID. Nitrogen was used as carrier gas at a flow of 1 kg/cm^2. The temperature of oven, injector and detector were 230, 270 and 300℃, respectively. Quantitation was carried out by means of external standard method.

Calculatioin of Bioconcentration Factor and Depuration Rate Constants Bioconcentration factor (BCF) was calculated from the following equation :

$$BCF = \frac{\text{pesticide concentration in whole body of fish}(\mu g/g)}{\text{pesticide concentration in water}\ (\mu g/ml)}$$

The pesticide concentration in the water at each sampling time was used for the calculation of BCF.

The following equation was used for the calculation of depuration rate constants of pesticides from the whole fish body :

$$C = C_0\ e^{-kt}$$

where

C = pesticide concentration in whole body of fish(㎍/g) at time, t,

C_0 = initial pesticide concentration in whole body of fish(㎍/g),

k = depuration rate constant(hr-1),

t = time(hr).

Ⅲ. Results and Discussions

1. Acute Toxicity of Methidathion and Phosalone

The values of 24-hr LC_{50}, 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50} were 28.34, 25.98, 24.43 and 22.03 ㎎/ℓ for methidathion, 3.76, 2.43, 1.86 and 1.05 ㎎/ℓ for phosalone, respectively(Table 1). The LC_{50} value in zebrafish showed that acute toxicity of phosalone was higher than that of methidathion.

Table 1. Acute toxicity of methidathion and phosalone to zebrafish

Pesticides	LC_{50}(㎎/ℓ)			
	24 hr	48 hr	72 hr	96 hr
Methidathion	28.34	25.98	24.43	22.03
Phosalone	3.76	2.43	1.86	1.05

2. Bioconcentration and Depuration of Methidathion and Phosalone

Plots of bioconcentration and depuration of two pesticides are shown in Fig. 1, 2, 3 and 4. The concentration of methidathion in zebrafish reached an equilibrium in 12 hours at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(low and high concentrations). The average BCF values of methidathion were 11.25(n=4) and 8.72(n=4) at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(low and high concentrations) after 12～72 hours.

Depuration rate constants and half-life of methidathion were not estimated at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(0.022 mg/ l and 0.22 mg/ l) because depuration rate of phosalone was very fast within 6hrs.

The concentration of phosalone in zebrafish reached an equilibrium in 12 hours at one-hundredth concentration of 96-hr LC_{50}(high concentration). The average BCF value of phosalone was 48.88(n=4) at one-hundredth concentration of 96-hr LC_{50}(high concentrations) after 12～72 hours. However, phosalone was not detected throughout the experimental period at the low concentration(0.001 μg/ml).

The concentrations of phosalone in zebrafish at one-hundredth concentration of 96-hr LC_{50} decreased after 6 hours(0.18 μg/g). Depuration rate constant and half-life of phosalone were 0.17 hr-1 and 4.01 at high concentrations (0.01 mg/ l), respectively. Depuration rate constant and half-life of phosalone were not estimated at low concentrations (0.001 mg/ l) because

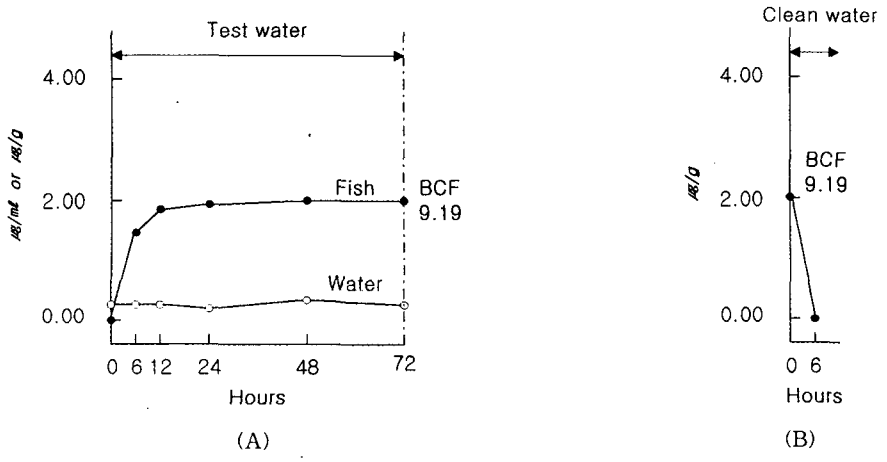

Fig. 1. Intake(A) and depuration(B) of methidathion (high concentration) by zebrafish.

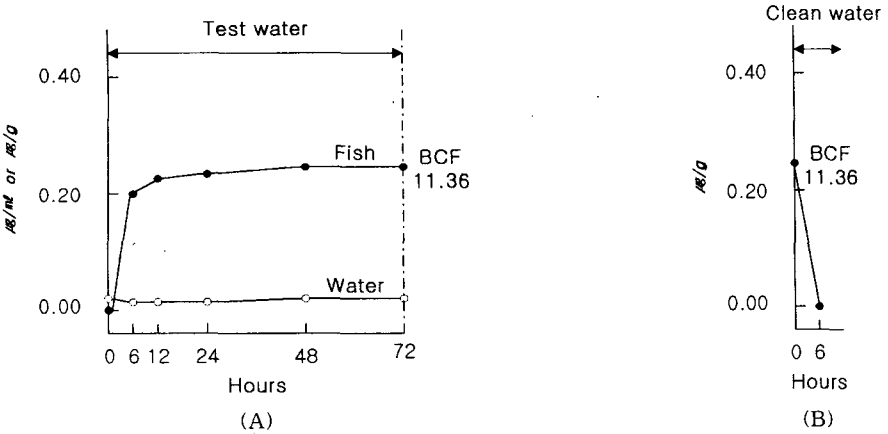

Fig. 2. Intake(A) and depuration(B) of methidathion (low concentration) by zebrafish.

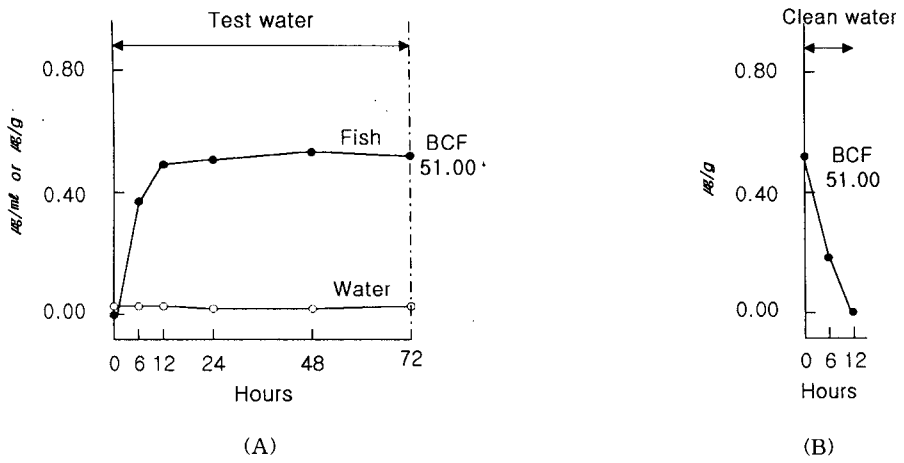

Fig. 3. Intake(A) and depuration(B) of phosalone (high concentration) by zebrafish.

depuration rate of phosalone was very fast within 6 hrs.

In the present study, acute toxicity of phosalone was higher than that of methidathion. The BCF values of phosalone were 5 times higher than those of methidathion, and depuration rate of methidathion was faster than that of phosalone.

It was suggested that the low BCF of methidathion was due to its very rapid depuration and very high water solubility (200 mg/ *l* at 20℃), and low lipophilicity (log Kow=2.20).[1] Therefore, the bioconcentration possibility of methidathion is not likely to occur in the environment. The determined BCF of phosalone was much higher than that of methidathion. The reason is that phosalone has relatively slow depuration and low water solubility (1.7 mg/ *l* at 20℃), high lipophilicity (log Kow=4.30) than those of methidathion.[1]

Ⅳ. Conclusions

The present study was performed to investigate the bioconcentration of methidathion and phosalone. The BCFs (bioconcentration factors), depuration rate constants and LC_{50} for two pesticides in zebrafish (*Brachydanio rerio*) were measured by the flow-through system (OECD guideline 305).

The 24-hr LC_{50}, 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50} were 28.34, 35.98, 24.43, 22.03 for methidathion. The concentration of methidathion in zebrafish reached an equilibrium in 12 hrs at low and high concentrations(0.022 mg/ *l* and 0.22 mg/ *l*). The average BCF values of methidathion were 11.25(n=4) and 8.72(n=4) at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(0.022 mg/ *l* and 0.22 mg/ *l*) after 12～72 hrs. Depuration rate constants and half-life of methidathion were not estimated at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(0.022 mg/ *l* and 0.22 mg/ *l*) because depuration rate of methidathion was very fast within 6 hrs.

The 24-hr LC_{50}, 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50} were 3.76, 2.43, 1.86 and 1.05 mg/ *l* for phosalone. The concentration of phosalone in zebrafish reached an equilibrium in 12 hrs at one-thousandth and one-hundredth concentration of 96-hr LC_{50}(0.001 mg/ *l* and 0.01 mg/ *l*). The average BCF value of phosalone was 48.88(n=4) at one-hundredth concentration of 96-hr LC_{50} (0.01 mg/ *l*) after 12～72 hrs. However, phosalone was not detected throughout the experimental period at the low concentration (0.001 μg/ml). Depuration rate constant and half-life of phosalone were 0.17 hr-1 and 4.01 at high concentrations(0.01 mg/ *l*), respectively. Depuration rate constant and half-life of phosalone were not estimated at low concentrations(0.001 mg/ *l*) because depuration rate of phosalone was very fast within 6 hrs.

The LC_{50} value in zebrafish showed that acute toxicity of phosalone was higher than that of methidathion. The BCF values of phosalone were 5

times higher than those of methidathion, and depuration rate of phosalone was faster than that of methidathion.

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